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IMMOBILIZATION OF GLUCOAMYLASE ON MACROPOROUS SPHERES

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Glucoamylase was covalently immobilized through the spacer-arm of the poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) spheres by using a glutaraldehyde as a coupling agent. The influence of the enzyme load, applied to the support on immobilization, yield and specific activity, has been determined. Obtained specific activity was 700 U/g with immobilization yield of 35 %. The K_m value for immobilized glucoamylase was 1.28 % (w/v), pH and temperature optimum were 4.5 and 70°C, respectively. The conversion of 20 % (w/w) starch hydrolysate achieved with the immobilized glucoamylase was 97 % after 5 hours.

KEYWORDS: Glucoamylase; immobilization; poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate)

INTRODUCTION

Immobilized enzymes are still in the focus of studies carried out in many different branches. At present, a lot of effort is being employed to develop catalytic hydrolysis reactions using immobilized enzymes. Considerable interest has been shown in the use of immobilized enzyme technology for production of dextrose from starch (1-3). An indispensable enzyme in starch hydrolysis is glucoamylase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3). Glucoamylase effects the hydrolysis of starch proceeding from the non-reducing end of the starch molecule to split off single glucose units at the α-1,4 linkages or at the α-1,6 branch points. To eliminate the disadvantages present in the conventional process, amyloglucosidase (AMG) was immobilized on various insoluble carriers with the re-

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tention of its catalytic properties which can be used repeatedly (4-6). The combination of unique biocatalytic properties of enzymes on one side, and heterogeneity of the support on the other, makes it possible to achive the main aim of immobilization, which is reusing the enzymes. Binding of enzyme to a support may be achieved by different methods. Some methods are based on the reaction between glutaraldehyde and amino-groups of the support (7-10). The enzymes are linked to an insoluble matrix by chemical bonds, generally rendering very stable derivates in which enzyme leakage is prevented. Immobilization of enzymes through covalent attachment has also been demonstrated to induce higher resistance to temperature, denaturants, and organic solvents in several cases (11, 12).

The nature of the matrix is a very important parameter when evaluating the immobilization process. Support materials which play an important role in the utilisation of an immobilized enzyme should be non-toxic, should have a good mechanical and chemical stability and provide a large surface area suitable for enzyme reactions. Poly (glycidyl methacrylate-co-ethylene glycol dimethacrylate) (poly(GMA-co-EGDMA)), due its appropriate feature, is a suitable matrix for immobilization of enzymes (13).

A new carrier for immobilizing glucoamylase has been prepared, and its properties were investigated herein. The present study deals with the immobilization of glucoamylase on macroporous spheres of poly(GMA-co-EGDMA) and examination of kinetic parameters of immobilized enzymes. Finally, the application of immobilized glucoamylase in an enzyme reactor is presented.

EXPERIMENTAL

Polymer

Macroporous copolymer of glycidyl methacrylate and ethylene glycol dimethacrylate (poly(GMA-co-EGDMA)) was prepared by suspension polymerization as previously described (14). For further experiments a polymer with particle size of 150-500 μm was used.

Polymer modification

The polymer was modified with 1 mol/l 1, 2-diaminoethane at 60°C for 4 h at pH 10. After that the polymer was washed several times with water and concentration of amino groups was determined by titration with 0.01 mol/l HCl in 0.5 mol/l KCl.

Enzyme

Industrial glucoamylase (amyloglucosidase; exo-1,4-α-glucosidase; EC 3.2.1.3 from *Aspergillus niger*) was supplied from Mapol (Warszava, Poland). The specific activity of the lyophilized enzyme was 85 U/mg of solid. Glucoamylase was assyed as follows. The reaction mixture contained 0.490 ml of 4 % (w/v) soluble starch in 0.05 M/l sodium acetate buffer (pH 4.5) and 0.010 ml of enzyme solution. The reducing carbohydrates formed were quantified using dinitrosalicylic acid reagent (15). One enzyme unit was defined as the amount of enzyme which releases reducing carbohydrates equivalent to 1 μmol glucose from soluble starch in 1 min at pH 4.5 and at 60°C.

Glucoamylase immobilization

The amino group activation step was done in 0.05 M phosphate buffer, pH 7.0, with 2.5% glutaraldehyde (16). After activation the carrier was washed off and then suspended in 1mg/mL of glucoamylase solution in 0.05 M acetate buffer pH 4.5. The excess enzyme was washed off with buffers: 0.05M acetate pH 4.5, 0.05 M acetate buffer with 1M NaCl, pH 4.5. The bound activity was defined as the difference between the activity in glucoamylase solution in which carrier was suspended and activity recovered in the washings. The immobilized enzyme was finally stored in acetate buffer pH 4.5 in 4°C until use.

Properties of enzyme preparation

The reaction mixture contained an appropriate amount of suction dried immobilizate (about 50 mg) and 10 ml of 4 % (w/v) soluble starch in 0.05 M/l sodium acetate buffer (pH 4.5). Obtained reducing carbohydrates were quantified by DNS method (15). The specific activity of immobilized enzyme was defined as number of micromoles of glucose which was liberated in one minute by one gram of dried immobilizate at 60°C.

The effect of temperature on the specific activity of the immobilized enzyme was determined by incubation at temperature ranging from 40 to 80°C with 4 % (w/v) soluble starch in 0.05 M/l sodium acetate buffer pH 4.5. The specific activity at 70°C was arbitrarily set as 100 % relative activity.

The optimal pH was determined with 4 % (w/v) soluble starch in 0.05M sodium citrate phosphate buffer of pH values varying from 3 to 7 at 60°C. The specific activity value obtained in pH 4.5 was taken as 100 % relative activity.

Batch reactor

Starch hydrolysate (SH) obtained with acid hydrolysis of starch with DE value of 65 ± 5 % was a gift from Jabuka (Pančevo, Serbia and Montenegro). Hydrolysis of SH was performed by incubating 10 ml of reaction mixture, 20 % (w/w) SH, in 0.05 M acetate buffer, pH 4.5 with 0.3 g of suction dried immobilizate at 40°C. The agitation speed was constant in order to eliminate the effects of external mass transport. Samples, 10 µl, were taken periodically to determine reducing sugars (15).

RESULTS AND DISCUSSION

Glucoamylase was covalently immobilized to macroporous spheres of poly(GMA-co-EGDMA). Concentration of amino groups after modification of polymer was 1.2 mmol/g. The immobilization yield, defined as the ratio of the specific activity of immobilized enzyme to the bound activity, decreases but the specific activity of the immobilized preparation increases when the amount of bound activity is increased Fig.1.

Specific activity of the immobilized glucoamylase reached a maximum at a bound activity of 2000 U/g dry polymer and that is the result of diffusional limitations caused by polymer that reduces the access of substrate to active sites of bound enzyme. According to this specific activity of 700 U/g dry polymer has a maximum activity of immobilized

preparate with yield of 35 %. In all further experiments, immobilized enzyme (IME) with the characteristics mentioned above was used.

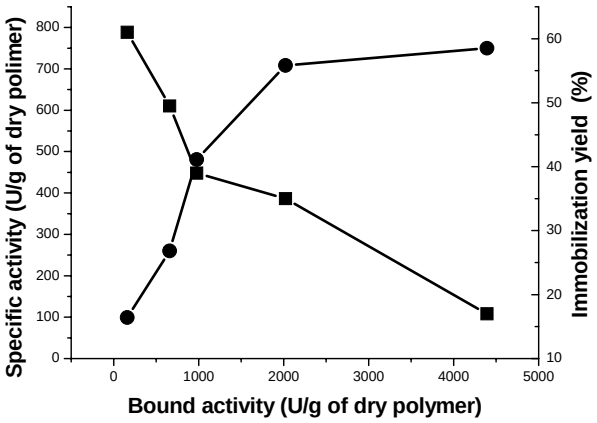

Fig. 1. Dependence of immobilization yield (■) and specific activity of immobilized enzyme (●) on bound activity

The obtained IME had a pH optimum of 4.5 (Fig. 2).

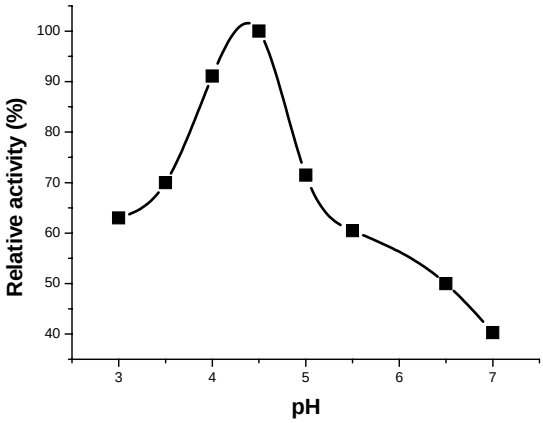

Fig. 2. pH dependence of IME activity for the hydrolysis of 4 % (w/v) soluble starch at 60°C

Immobilization did not change the optimum pH value when compared with soluble enzyme (1). From Fig. 3 it can be seen that the immobilizate had an optimal temperature for the hydrolysis of soluble starch at 70°C.

Immobilization of glucoamylase on polymer that we used increased temperature optimum for 10°C when compared with previously reported results (2). The obtained results

show that our IME could have a higher operation stability under the industrial conditions than reported before (2, 3).

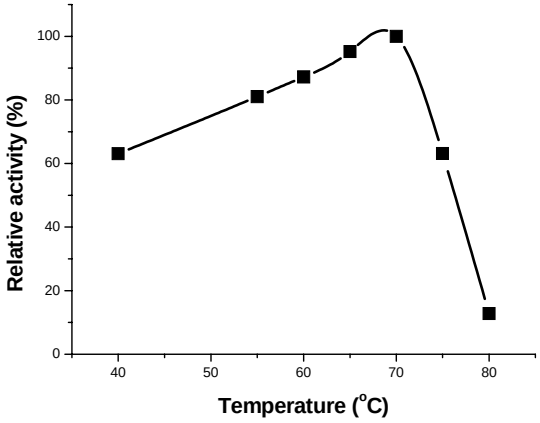

Fig. 3. Temperature dependence of IME activity for the hydrolysis of 4 % (w/v) soluble starch in 0.05 M/l sodium acetate buffer pH 4.5

K_m value for soluble starch was calculated from Lineweaver-Burk plot within the starch concentration of 0.1-4% (w/v). K_m value for immobilized glucoamylase on macroporous polymer was 1.28% (w/v) and it is very similar to the results reported previously when glucoamylase was immobilized on methyl methacrylate (17) but less than in the case of immobilization on an acrylic copolymer (18). The obtained K_m value is higher than for soluble enzyme because of diffusional limitations.

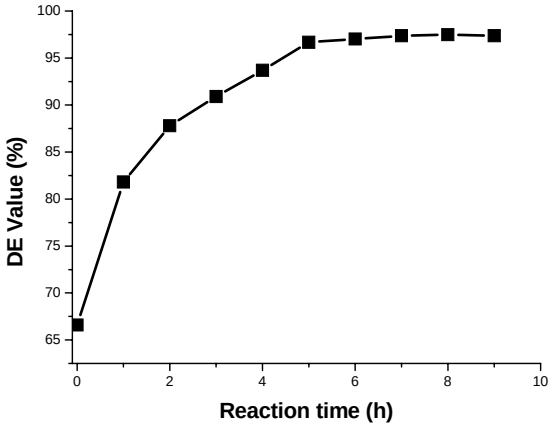

Fig. 4. Saccharification of 20 % (w/w) SH with immobilized glucoamylase. DE (dextrose equivalent) is a measure of the reducing power of the solution based on pure glucose as 100 and pure starch as 0

In order to investigate the obtained immobilized glucoamylase, hydrolysis of SH was carried out under conditions analogous to those used on a large scale in the industry. Fig. 4 shows the kinetics of SH hydrolysis in a batch reactor.

In the saccharification process of SH, the immobilized enzyme gave DE 97 % after 5 hours. This result is very similar to the one previously reported (18). Obtained DE value is in accordance to the value obtained with the soluble enzyme in the industrial saccharification of liquefied starch (19)**.** The substantial reduction in the reaction time is a result of the high enzyme loading per reactor volume.

CONCLUSIONS

The high specific activity attained during the coupling of glucoamylase on glycidyl methacrylate spheres, proved that this support meets many of the desired characteristics for enzyme immobilization.

The immobilization process gave IME with higher K_m and temperature optimum than the soluble one, while pH optimum did not change significantly. According to our experiments performed on a laboratory scale, the use of immobilized derivative of glucoamylase allowed a substantial reduction in the reaction time required for producing high-content glucose syrups batchwise (5-6 hours), which for the soluble enzyme, under the conditions used in industry, are approximately 48 to 72 h. The IME gave the same final dextrose equivalent as the soluble one.

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ИМОБИЛИЗАЦИЈА ГЛУКОАМИЛАЗЕ НА МАКРОПОРОЗНИМ СФЕРАМА

Ненад Б. Милосавић, Радивоје М. Продановић, Слободан М. Јовановић и Зоран М. Вујчић

Глукоамилаза је имобилизована преко спејсера на сфере кополимера глицидил метакрилата и етилен гликол диметакрилата уз помоћ глутаралдехида. Одређен је утицај количине додатог ензима на принос имобилизације као и на специфичну ак-

тивност добијеног имобилизата. Добијена је специфична активност од 700 U/g са приносом имобилизације од 35%. Км вредност имобилизоване глукоамилазе је 1,28 % (w/v), pH и температурни оптимуми су 4,5 и 70°C. Имобилизовани ензим је при хидролизи 20 % (w/w) хидролизата скроба постигао конверзију од 97 % након 5 сати.

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